



Particle characteristics of microplastics contaminating the mussel *Mytilus edulis* and their surrounding environments

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ABSTRACT

We investigated the environmental partitioning and particle characteristics of macro-, meso- and microplastics and their uptake into the mussel, *Mytilus edulis*. Sediment samples, overlying seawater and mussels from 9 intertidal locations in the South West of England were analysed for abundance and type of microplastic. Micro- and mesoplastic-like particles were found in 88.5% of the 269 mussels sampled, ranging from 1.43 to 7.64 items per mussel. Of these plastic particles, 70.9% were identified as semi-synthetic (mainly modified-cellulose). Mussel microplastic abundance, but not polymer type, was correlated with that of their surrounding sediment, but not with sea-surface microplastic concentration or mussel size for our study sites. We found significant differences in the relative abundance of polymer types and particle sizes between seawater, sediment, and mussels, with mussels over-representing modified-cellulose fibre abundance but under-representing polyvinyl. Mussels contained significantly smaller plastic fragments than their surrounding sediment and shorter fibres than their overlying seawater.

1. Introduction

There has recently been a dramatic rise in public awareness, policy and scientific focus on plastic waste, particularly in single-use consumer products and the role of microplastic as an environmental contaminant. Between 4.8 and 12.7 million metric tonnes of plastic are thought to enter the marine environment each year (Jambeck et al., 2015), resulting in an estimated 93–236 thousand metric tonnes of microplastic particles floating on the sea surface (van Sebille et al., 2015). Plastic pollution is a global issue, with macro and microplastics now known to be present throughout both freshwater and marine ecosystems from the Arctic, to the tropics and coral reefs, and the deep sea (Courtenes-Jones et al., 2017; Cózar et al., 2017; Hall et al., 2015). The definition of microplastic debris was originally arbitrarily proposed as any plastic particle < 5 mm, (Arthur et al., 2008) but it has recently been suggested this should be re-defined as particles 1 to < 1000 μm, with particles 1 mm to 10 mm now being referred to as mesoplastic (Hartmann et al., 2019). Plastic debris comprises a complex mixture of particles which are often categorised by visual characteristics such as size, colour, and shape, and is a relatively diverse pollutant, covering a wide range of sizes and shapes from larger litter items down to the

nano- scale, and a range of different buoyant and non-buoyant polymer types (Hartmann et al., 2019). Micro- and mesoplastic particles fall within the size range of the optimal prey species for many animals at the base of the marine food web (Galloway et al., 2017) with increasing evidence of their ingestion by a wide range of species from zooplankton (Desforges et al., 2015) to marine mammals (Nelms et al., 2019). This combined with their prevalence and persistence throughout marine ecosystems has raised concerns globally over their potential impacts to marine species.

Globally, coastlines are diverse habitats supporting an abundance of ecologically and economically important marine species. Coastal microplastic pollution has been shown to vary by region and is dependent on a wide variety of factors such as oceanic currents, local tides and geography (Jambeck et al., 2015), but typically microplastic concentrations are high, likely due to the constant land-based input. Although plastic pollution is ubiquitous in the marine environment and can travel long distances from its sources, localised sources such as wastewater effluent and poor waste management from coastal urban populations contribute a significant component of coastal microplastic pollution (Graca et al., 2017; Jambeck et al., 2015). Hence the risk of biological uptake of microplastics in coastal regions is thought to be

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relatively high (Clark et al., 2016; Graca et al., 2017). Whilst data on the sea-surface distribution and abundance of microplastics has increased greatly in recent years, our understanding of the movement of plastic particles away from the surface, through marine ecosystems and their ultimate fate in the marine environment remains limited. Processes such as biofouling, ingestion and subsequent incorporation into faeces, and eventual aggregation with organic matter (Zhao et al., 2018), all influence the buoyancy of plastic particles (Galloway et al., 2017) leading to the recent understanding that most plastic eventually sinks to the benthos (Koelmans et al., 2017). Hence, benthic sediments may be a major sink for plastic particles (Kaiser et al., 2017; Porter et al., 2018; Woodall et al., 2014). Along the coastline, where many benthic species feed, particles may also be re-suspended by turbulent currents and bioturbation, potentially keeping these microplastics bioavailable to benthic feeders. Microplastics are known to be readily ingested by a range of marine species including pelagic and benthic fish and invertebrates, hence benthic coastal species may be at greater risk from plastic contamination (Graca et al., 2017; Halstead et al., 2018; Lusher et al., 2013; Rummel et al., 2016). Understanding the local factors that influence biological uptake of microplastic by coastal benthic species is critical to being able to assess the risk that this pervasive pollutant poses to these important ecosystems (Seitz et al., 2014).

The mussel, *Mytilus edulis*, is a keystone coastal species with important roles in ecosystem functioning; including habitat formation for diverse benthic communities (Joint Nature Conservation Committee, 2008) and nutrient recycling. They play an important role in benthic-pelagic coupling by removing large quantities of suspended organic matter from the water by filter-feeding, and through the production of faeces and pseudofaeces (Ward and Shumway, 2004) and process large volumes of water; for example under optimal algal conditions a 21.5 mm sized mussel will filter an average of 15 mL min^{-1} (Riisgård et al., 2011). Coupled with their wide geographical range and low metabolic transformation rates, these traits make mussels useful in monitoring programmes as effective small stationary water samplers for many potential pollutants and dissolved chemical contaminants. The relationship between the level of waterborne contaminants and bivalve tissue concentrations is well established, for example in the NOAA Mussel Watch Programme which monitors over 150 organic and inorganic contaminants including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), the pesticide dichlorodiphenyltrichloroethane (DDT) (National Oceanic and Atmospheric Administration, 2018). It has been suggested by a number of studies that mussels can also be useful biomonitoring tools for evaluating environmental microplastic pollution (Beyer et al., 2017; Brate et al., 2018; Li et al., 2019), however the properties of particles within the definition of “microplastic” are complex and hence their uptake into biota from the environment may not follow the same relationships or behaviours as dissolved chemicals and or their derivatives.

Microplastic uptake by mussels is well established, both in laboratory studies (Browne et al., 2008; Van Cauwenberghe et al., 2015) and in their natural habitats and may occur by ingestion or adherence to tissues (Kolandhasamy et al., 2018; Qu et al., 2018), with numerous studies now reporting microplastic contamination of wild mussels (De Witte et al., 2014; Li et al., 2018; Li et al., 2016; Phuong et al., 2018; Qu et al., 2018; Santana et al., 2016; Zhao et al., 2018). Trophic transfer of microplastics ingested by mussels has also been demonstrated under laboratory exposure scenarios, providing a route through which microplastic particles can be accumulated and enter the food chain (Farrell and Nelson, 2013). Mussels are also economically important food species, accounting for more than a third (roughly 470 thousand tonnes) of production by weight of the aquaculture industry in the European Union (Eurostat, 2016). Hence microplastic ingestion by mussels is of additional concern for its human health implications in a species which we consume whole without removing the guts (Van

Cauwenberghe and Janssen, 2014).

Here, we investigate the environmental partitioning and particle characteristics of microplastics isolated from within mussels, with those of the micro- and mesoplastic particles of the mussels' immediate environment, via surveys of 10 mussel populations at 9 locations across the South West coast of the United Kingdom (U.K.). We include an assessment of the larger beach macroplastic debris at each location to assess whether there is any similarity in composition between the larger litter items and smaller microplastic items of beach plastic debris for each site. Understanding the environmental partitioning of the different types of plastic contamination across seawater and coastal sediments with its uptake in benthic mussels is key to assessing the risk that microplastic pollution poses to their ecological functions and their human consumers, as well as assessing their application as biomonitoring tools for microplastic pollution.

2. Materials and methods

2.1. Site selection

Sampling took place at 9 locations on the South West coast of U.K. during the August–December period in 2017 (mussels only) and 2018 (seawater, sediment and mussels, see SI Fig. S1 for a map of the locations and their latitude and longitude). Crooklets beach, Barricane beach, Constantine Bay, and Port Gaverne were sampled in 2017; Starcross, Yelland Quay and Trebarwith Strand were sampled in 2018. Torquay and Whitsand Bay were sampled in both 2017 and 2018. The sampling sites are mostly rocky shore beaches, with the exceptions of Starcross and Yelland quay which are estuarine habitat on the river Exe and Taw estuaries, respectively.

2.2. Water sampling

Surface seawater was sampled in triplicate for each site using a $53 \mu\text{m}$ plankton net, towed through surface water for 3 min within 10 m of the waterline, at a minimum depth of 25 cm to allow full submersion of the net. All other samples were filtered to $50 \mu\text{m}$ so as to have a consistent limit of detection for all environments sampled and hence make our results comparable across sediment/mussel/water compartments. GPS coordinates were recorded at the start and the end points of each trawl (Garmin GPSMAP® 78s) to calculate the distance of the trawl. The contents of the net were then thoroughly rinsed into 0.5 L Nalgene sample bottles using MilliQ, ultra-pure water filtered to $0.22 \mu\text{m}$ to avoid contamination from rinse water. Samples contained suspended sediment and organic matter which was allowed to settle in the bottles, then the supernatant was then filtered through $50 \mu\text{m}$ polyamide nylon mesh (Plastok® Associates Ltd.) using a vacuum filter in a laminar flow hood to reduce atmospheric contamination. Microplastic-like particles were removed from this sediment by ZnCl_2 density floatation separation, using the method for sediment analysis detailed below, then filtered through the same mesh as the respective supernatant. Filters were stored in sealed square petri dishes until analysed (below).

2.3. Sediment collection and density separation

Three sediment samples were collected at each site, one from within the strand line, one from the middle of the beach, and one close to the low tide mark. Sediment was collected adjacent to the mussel beds by taking the surface 1 cm of sediment from within a 1 m^2 square quadrat with a metal trowel. Sediment samples were then stored in clean plastic sample bags at -20°C until analysed. Defrosted sediment was placed into 1 L beakers and then into a drying oven at 60°C overnight. From each of these samples (three per site), a further three 50 g sub-samples of dry sediment were then taken for the isolation of microplastics, resulting in a total of 450 g of analysed sediment per sampling site. Whilst

this is a relatively small amount of sediment to analyse per site, this allows the use of Sediment-Microplastic Isolation (SMI) units, custom-built according to the design and methods developed by Coppock et al. (2017), to separate potential microplastics from the sediment with a high recovery efficiency (95.8%). This technique allows better recovery of micro- and meso-sized particles. A pre-filtered (50 μm) ZnCl_2 solution at a density of 1.5 g cm^{-3} , was chosen as a floatation media based on its effective recovery of dense polymers. The ZnCl_2 sediment solution was filtered through 50 μm polyamide nylon mesh using a vacuum filter and stored in sealed square petri dishes until analysed (below).

2.4. Mussel sampling

Thirty mussels were collected from each site (269 sampled in total, mean length $41.6 \text{ mm} \pm \text{SD } 12.7$, 29 mussels from Starcross) selected to cover a wide range of mussel sizes, positions and orientations of the mussels on the substrate and within the site. Mussels were stored in plastic sample bags, and stored in a freezer at -20°C until dissection. All subsequent work was carried out inside a laminar flow hood to minimise airborne contamination with a clean filter paper placed in a petri dish to collect airborne contamination. Once defrosted, the width and length of the shell of each mussel was measured and then thoroughly rinsed with MilliQ to remove external microplastic contamination. Mussel soft tissue was then excised and wet weight measured. During this process samples were covered with foil to avoid airborne contamination. Mussel tissue was then digested at 70°C oven in 10% potassium hydroxide until fully digested, up to 48 h (within the range of conditions used in previous studies, reviewed by Lusher et al. (2017)). The contents of each sample were filtered through 50 μm nylon mesh (for consistency with the seawater and sediment limit of detection) using a vacuum filter. Filters were stored in sealed petri dishes until further analysis.

2.5. Beach litter survey

Large plastic items were collected within a 100 m section of the beach, from the low tide mark to the back of the beach. All visible plastic was collected within an upper time limit of 90 min and standardised to the number of participants involved. Collected items were categorised using the OSPAR guideline for monitoring marine litter on beaches (OSPAR Commission, 2010b). We removed 10% of items of each category, minimum of 1 item, for FT-IR spectrometry analysis.

2.6. Analysis of filters and FT-IR analysis

Filtered material was analysed visually using a dissecting microscope at $30\times$ magnification. Potential microplastic particles were counted and classified by shape and colour, and 10% of each category, with a minimum of three particles, were removed and stored for spectral analysis. To account for any contamination of laboratory origin, procedural blanks were performed (6 per site for mussel, and 1 per site for water and sediment samples) that underwent the same processing as water, sediment, and mussel samples but did not contain a sample. On analysis, blank samples included only fibrous particles, which is likely airborne contamination from clothing. Mussel sample blanks contained on average 1.86 ± 0.28 black fibres, 1.62 ± 0.33 clear fibres, and 0.12 ± 0.05 red fibres. The mean number of particles for each particle category (shape and colour) across the blanks was subtracted from all data prior to data further analysis and is not included in any data presented.

Potential microplastic particles were analysed using a PerkinElmer Frontier Fourier-transform infrared (FT-IR) spectrometer. For larger pieces that could be easily handled, FT-IR analysis was carried out using a universal diamond –ATR attachment. For the majority of smaller pieces FT-IR spectra were obtained using a PerkinElmer Spotlight 400 $\mu\text{FT-IR}$ Imaging System (MCT detector, KBr window) operating in

reflectance mode and with a wavenumber resolution of 4 cm^{-1} . A total of 16 scans were collected, across a wavenumber range from 4000 to 650 cm^{-1} . Spectra were then processed using Perkin-Elmer's Spectrum™ 10 (version 10.5.4.738), enabling normalisation of the data and base-line correction. Polymers were identified by automated matching against commercially available spectral libraries, including Perkin-Elmer's standard Polymers Library. Only match qualities $> 70\%$ were accepted, with an average match quality of samples of 85%. Particles were photographed using the spectrometers imaging software and the lengths of fibres and fragments then measured using ImageJ 1.47v (Schneider et al., 2012). Prior to data analysis, particle categories which could not be confirmed as synthetic by $\mu\text{-FTIR}$ spectrometry, were excluded. This included “film” in which all particles examined were confirmed as chitin, and “white beads” which were all confirmed as calcium carbonate mussel pearls. Larger plastic pieces from the beach litter survey were analysed using a Cary 630 FTIR spectrometer (Agilent Technologies). Samples were prepared for analysis by removing the degraded and biofouled surface layer with a razor blade to improve the quality of the spectra. Biofilms have been shown to mask the distinct identifying peaks of synthetic polymers (Ghosal et al., 2018).

2.7. Data analysis

Data presented is based on the confirmed anthropogenic particles following FTIR analysis. Statistical analyses (ANOVA and linear regression) were performed on data corrected for contamination found in procedural blanks using SPSS Statistics 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY). Differences between total number of particles in seawater, sediment, and mussel samples were determined using One-Way ANOVA with a Tukey's post hoc test. Linear regressions were used to determine the relationship between microplastic in mussel tissue, seawater and sediment. Linear regression was also used to determine the relationship between mussel size and microplastic particle abundance. Statistical significance was accepted at $p\text{-value} < 0.05$.

3. Results and discussion

Microplastic contamination of seawater, coastal sediments, and mussels was evident at all of our sampling locations across the South West of the U.K. All surface seawater samples contained microplastic particles, with concentrations ranging from 1.97 to 3.38 particles m^{-3} , but with no significant differences in seawater concentrations of these particles across our study sites (Fig. 1a, one-way ANOVA, $F_{4,10} = 0.228$, $p\text{-value} = 0.916$). Of these floating particles, 51% were microfibrils and 47% were fragments, with only 0.03% comprising microbeads. Microplastic contamination of the surface layer of intertidal sediment did differ significantly between locations (Fig. 1b, one-way ANOVA, $F_{4,10} = 4.544$, $p\text{-value} = 0.024$), with concentrations ranging from 33.9 particles kg^{-1} at Torquay to 402.0 particles kg^{-1} at Whitsand Bay. The majority of these particles were microfibrils (93%), with only 7% being fragments, found in samples from only three of the five sites analysed for sediment. No microbeads were observed in the sediment samples analysed from our study sites.

Microplastic particles were found within 238 of the total 269 mussels sampled (i.e. 88.5% of mussels) across the 10 mussel populations studied (from 9 locations; two different populations were sampled within Torquay Bay) (Fig. 1c). Whilst seawater microplastic concentrations did not differ across sites, the particle load per mussel did differ significantly between our study sites (One-way ANOVA; $F_{9,259} = 4.018$, $p\text{-value} < 0.001$ Fig. 1c), with mussels from Whitsand Bay containing the highest average particle loads of 7.64 ± 1.61 particles per individual, and Torquay (harbour) the least, with 1.43 ± 0.30 particles per individual. Of these particles, 87% were microfibrils whilst 12% were fragments. Only 9 microbeads were found

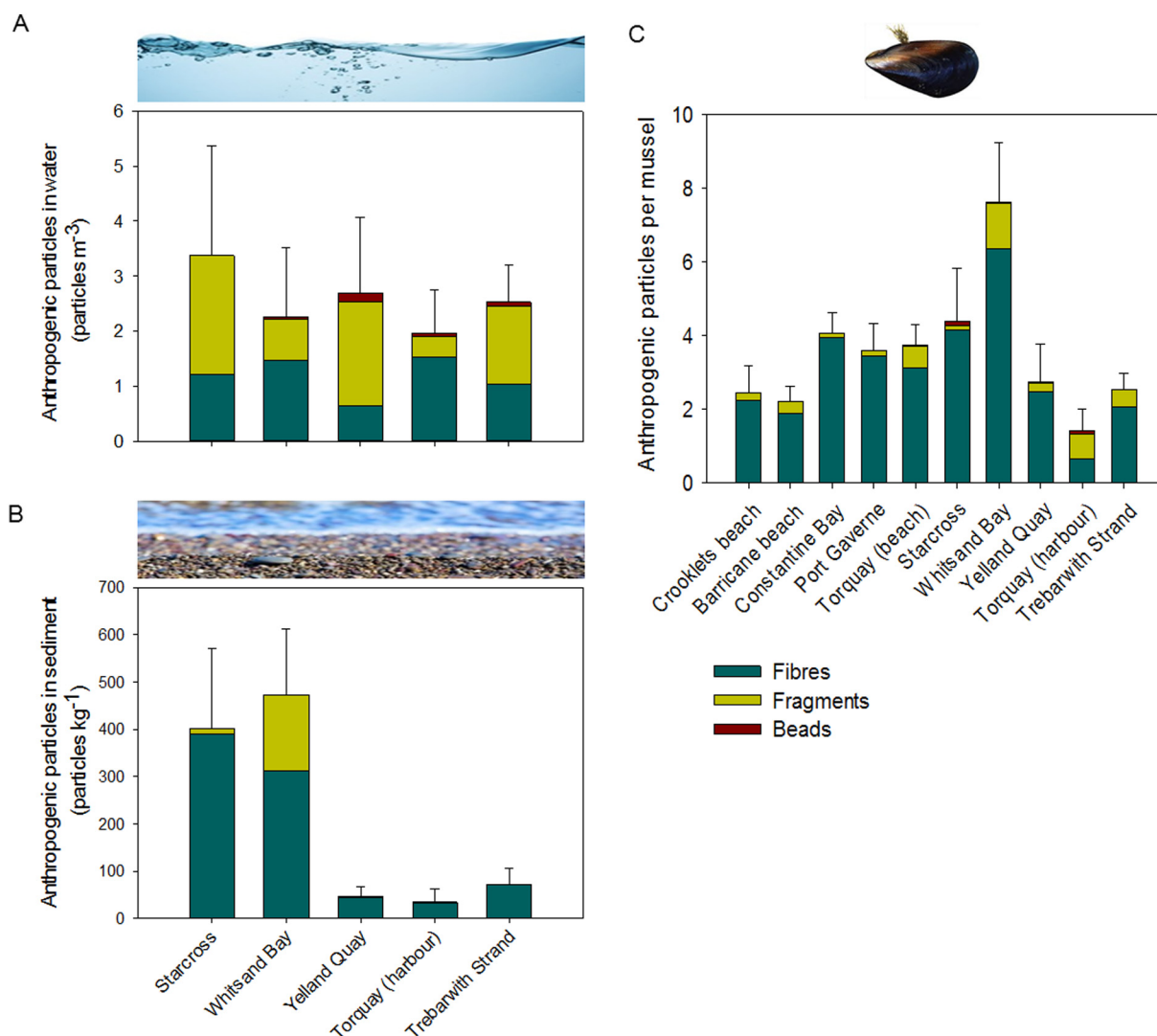


Fig. 1. The average number of microplastic-like particles, characterised according to shape found in (A) surface seawater (2018 data) (B) the surface 1 cm of sediment (2018 data) and (C) within the tissues of the mussel *Mytilus edulis* (2017 and 2018 data) at coastal sites in Devon and Cornwall, SW England. Data as mean \pm standard error (limit of detection cross all samples of 50 μ m).

within mussels across all sites sampled ($< 1\%$). These numbers of microplastic particles per individual mussel are similar to the range reported in a previous study on microplastic contamination of mussels in the U.K. (Li et al., 2018) (1.1–6.4 items per individual) and are similar to those reported in China (Li et al., 2018) and Norway (Brate et al., 2018). However they are higher than the contamination levels reported for mussels in other studies from Belgium, Germany, French and Dutch coastal waters (De Witte et al., 2014; Van Cauwenberghe et al., 2015; Van Cauwenberghe and Janssen, 2014). The highest numbers of microplastics reported for mussels to date is that reported for mussels collected from a beach in Nova Scotia, Canada, where 34–178 items/individual was recorded, mostly comprising microfibrs (Mathalon and Hill, 2014).

Micro-FTIR spectroscopy was conducted on 247 randomly selected particles from across the seawater, sediment and mussel samples. This analysis revealed that 33.9% of these particles were synthetic plastic polymers, mainly polystyrene, polyethylene and polypropylene (Fig. 2). Particles of natural origin, 9.3% of items analysed, and spectra with a low match quality (below 70%) were discarded from our final results and are not presented in our data. A large number of particles (56.8%), were semi-synthetic fibres comprised of modified-cellulose. Potential rubber fragments were also found in some samples but are not included

in the data presented due to difficulties in generating high quality FTIR spectra from these particles. The modified-cellulose fibres were mostly black/blue or red and hence are likely to be viscose/ rayon fibres from textiles, therefore we include these within our counts as these highly modified natural polymers have been included within the recent ‘microplastic’ definition suggested by Hartmann et al. (2019) due to their artificial composition.

This follows an emerging trend for studies in coastal areas where particles are subsequently analysed using μ FT-IR or other spectral techniques such as Raman, which often find a high percentage of anthropogenic particles in seawater or ingested by marine species comprise modified-cellulose-based anthropogenic materials such as viscose or rayon (Remy et al., 2015), or natural fibres such wool or cotton (Courteney-Jones et al., 2017; Halstead et al., 2018; Li et al., 2018). For example, Brate et al. (2018) reports cellulose fibres as the dominant particle in mussels on the Norwegian coast, whilst a recent global study found that 57% of the microfibrs isolated from marine samples are classified as synthetic, 12% as semi-synthetic, and 31% as non-synthetic (Barrows et al., 2018). According to the recent Hartmann et al. (2019) review, synthetic-cellulose fibres should be considered within the definition of ‘plastic debris’ due to their highly modified and persistent nature, however distinguishing between synthetic and natural

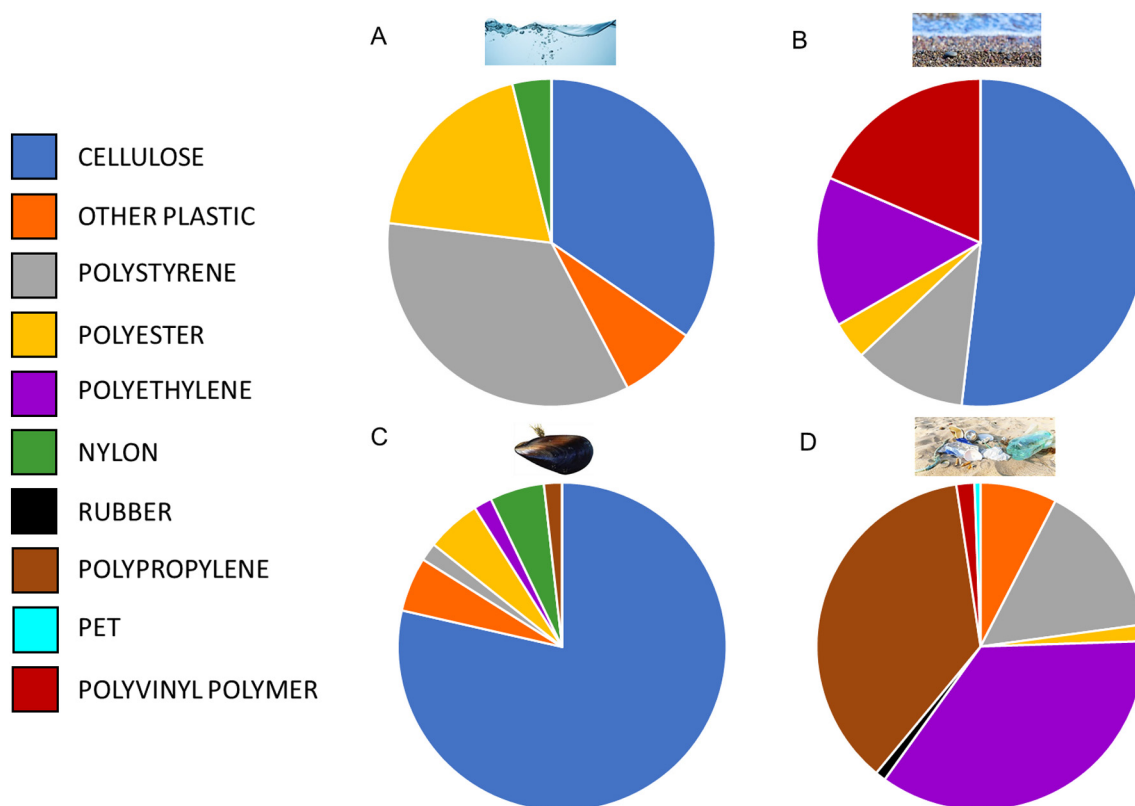


Fig. 2. Results of ATR/FT-IR spectral analysis, showing proportions of polymers of anthropogenic particles present in (A) samples of seawater, (B) the surface 1 cm of sediment, (C) within *Mytilus edulis*, and (D) macroplastic beach debris from coastal sampling sites in Devon and Cornwall, SW England.

cellulose-based fibres using currently available μ FT-IR spectral libraries can be challenging, making categorising these fibres as either plastic or non-plastic particles problematic.

A variety of synthetic and semi-synthetic polymers were found across the different environmental compartments that we studied (i.e. seawater, sediment, mussels), however these were not all distributed equally across compartments, i.e. environmental partitioning of polymer types was observed which may influence what is bioavailable to a benthic mussel to ingest. For example, modified-cellulose made up significantly more of the particles found in mussels than in the overlying seawater or the beach litter at our sample sites (One-way ANOVA; $F_{3, 22} = 19.282$, p -value < 0.000, Fig. 2). The buoyant polymer polyester (7.5% of total) made up a significantly greater proportion of particles in the overlying seawater than those in the sediment or in the beach macroplastic items (One-way ANOVA; $F_{3, 22} = 5.990$, p -value = 0.004, Fig. 2b). There was a significantly greater proportion of polyvinyl polymers in the sediment than in mussels or the overlying seawater (One-way ANOVA; $F_{3, 22} = 8.039$, $p = 0.002$). Other polymers identified include polystyrene (11.0%), polyethylene (3.4%), polyvinyl-based polymers (4.2%), nylon (2.5%), modacrylic (1.7%), and polypropylene, polyacrylamide, ethylene/acrylic acid, and plasticizer (0.85% each) (Fig. 2a, b, c).

Macroplastic pollution of the strandline and intertidal zone was evident at all of our sampling locations but varied greatly in abundance from site to site. We collected a total of 7411 beach macroplastic debris items, of which 3723 items were collected from Whitsand Bay, accounting for more macroplastic items than the sum of all other locations. Trebarwith Strand was the least littered site with only 17 items collected. Macroplastic beach litter was diverse in composition but was dominated by fragmented plastic debris with pieces 0–2.5 cm and pieces 2.5–50 cm making up 44.8% and 35.0% of total collected items by number, respectively, consistent with previous beach litter studies for the U.K. (Nelms et al., 2017; Watts et al., 2017). Other items (< 5%

each) were mostly consumer products such as food and cosmetic item packaging and containers, ropes, cigarette lighters, and plastic bags (for full list of items see Table S1 in Supplementary materials). We analysed 811 of these macroplastic items using FTIR, with an average certainty of 85.4% in order to compare these polymer types with the composition of the microplastic particles found at the same locations and within the mussels. Despite a large variety in litter items, the macroplastic was dominated by only two buoyant polymers, polyethylene (35.4%) and polypropylene (36.6%), representing a significantly greater proportion than found at the micro-scale (One-way ANOVA; $F_{3, 22} = 7.747$, p -value = 0.001; $F_{3, 22} = 20.814$, p -value < 0.001, respectively, Fig. 2d). The remaining items comprised polystyrene (15.3%), polyvinyl polymers (1.78%), polyester (1.63%), rubber (1.0%), polyethylene terephthalate (0.6%) and ‘other’ plastic polymers (7.6%), (Fig. 2d).

These large differences in the polymer composition of large macroplastic litter on beaches and the microplastics found in the same sediments, the nearby surface seawater and within the mussels suggests that there is no direct relationship between the two size fractions of debris at the sites tested here, i.e. the larger macroplastics litter items are not the source of the smaller items on the same beach. The local coastal topography, sediment type, and hydrodynamics, in addition to particle characteristics are all likely to play a role to produce the mix of plastic items that accumulate on any section of coastline (Zhang, 2017). The fragmentation of coastal macroplastic debris might produce particles with altered physical characteristics from the original larger items which are then influenced differently by local physical factors. Particle shape, size, and density may determine a particles position in the water column and changes to these characteristics could determine the way in which the particles are transported (Kowalski et al., 2016; Lebreton et al., 2018). Whether a particle is in suspension or settled in the sediment could determine to what degree it affected by surface currents and turbulence, wind and wave induced drift, or benthic sediment

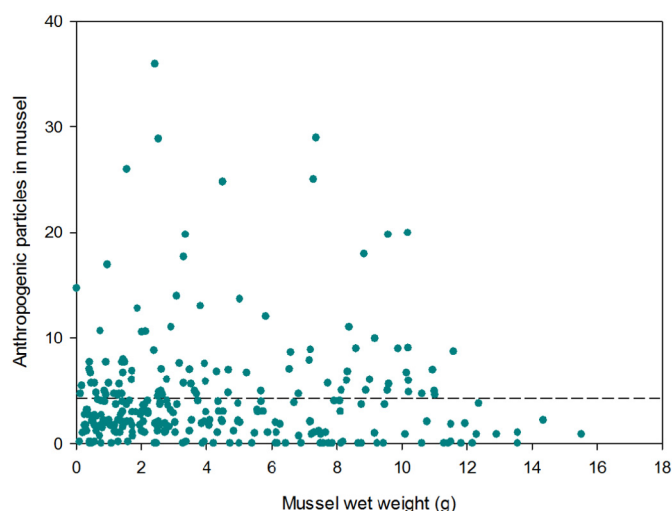


Fig. 3. Total anthropogenic particle load per mussel plotted against mussel wet weight (g) for all mussels sampled from all sites from both years (2017 and 2018). Regression line shown as dashed line. (Linear regression, $R^2 < 0.001$, $F(1, 267) = 0.001$, p -value = 0.976).

transport dynamics such as bed-load or suspended-load (Ballent et al., 2013; Zhang, 2017). Ultimately, changes to a particle's physical characteristics could result in transport away from the site of origin. This may explain why we find such a high percentage of polypropylene and polyethylene at the macro- scale, but not at micro- or meso-scales at these intertidal sites.

We found no correlation between the total number of anthropogenic particles in individual mussels and mussel wet weight (g) or any other parameter of individual size tested at the sites studied for this work (Fig. 3). This is in contrast with previous findings of Brate et al. (2018) who did find a relationship between mussel size and number of particles ingested in their study of Norwegian mussel populations. Studies in microplastic uptake often attempt to normalise their measures of plastic particles per individual by mass, following an assumption that size influences uptake rates in a similar way to respiration rates (Hamburger et al., 1983) and feeding rates (Riisgård et al., 2014). Our study suggests that this idea of scaling of microplastic uptake proportionally to size might not always hold true for *M. edulis* at this particle size range and lower concentrations of plastic contamination. Whilst mussel condition varies seasonally and hence shell length may be considered a more reliable indicator of filtration rate than tissue weight (Riisgård et al., 2014), we similarly found no relationship between shell length and microplastic uptake. Little dose response data exists for microplastic uptake for any marine species, particularly at these lower

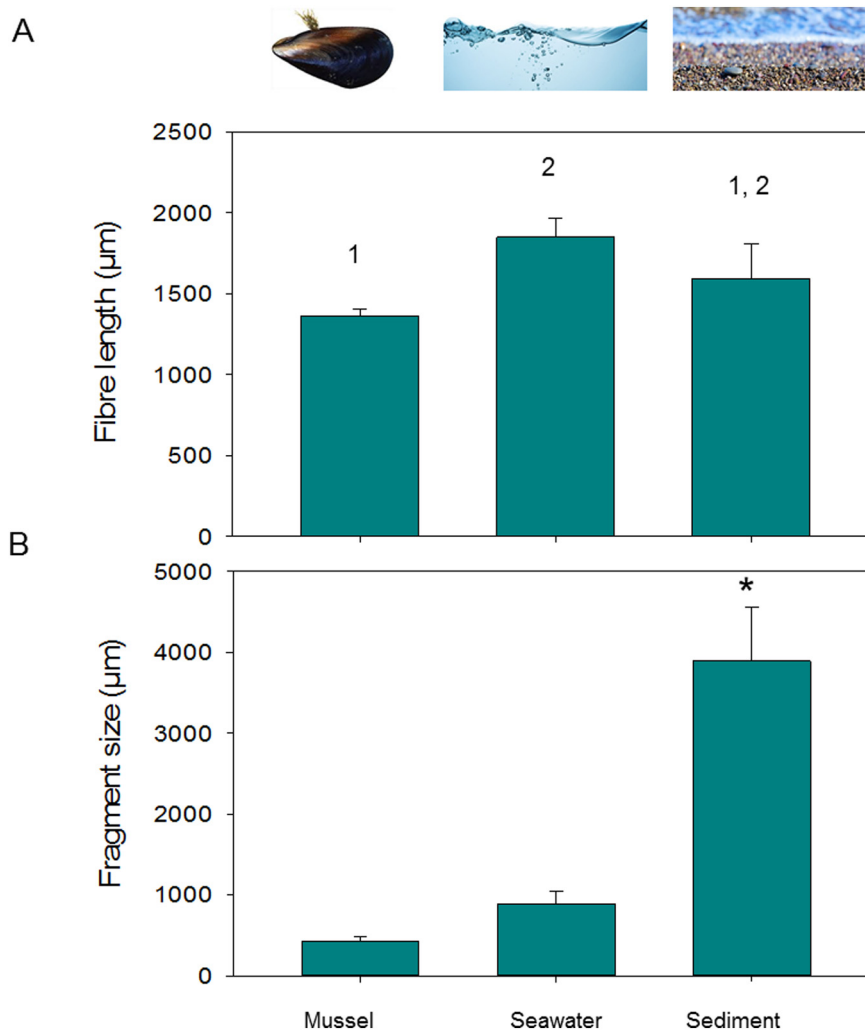


Fig. 4. Comparisons of the sizes of the two major categories, (A) fibres and (B) fragments, of observed anthropogenic particles in samples of *Mytilus edulis*, seawater, and the surface 1 cm of sediment from coastal sites in Devon and Cornwall, SW England in 2018. Groups labelled with the same number are significantly different. (One-way ANOVA; 1) p -value < 0.001, 2) p -value < 0.001, 3) p -value < 0.001).

environmental concentrations. Microplastics can also pass through guts and be egested with the faecal material (Cole et al., 2016), so may only ever be present within an individual for a short time related to the gut passage time of that individual. This coupled with the transitory nature of floating plastic debris creates a series of interdependencies when considering how mussels become contaminated with microplastics. For instance, microplastics have been found to vary by 3 orders of magnitude difference between sites only 32 km and 75 km away from a net tow within a 24 h period (Law et al., 2014) and therefore our data do not constitute a definitive view of the question at hand. This may be shape dependant with fibres potentially being more likely to be retained but the evidence supporting this idea is currently limited. Hence the dynamics of particle uptake and body load may not scale with size at these low concentrations but rather be driven by particle encounter rates influenced by localised seawater movement at micro-scales.

The size and shape of anthropogenic particles also appears to influence their uptake into mussels. We found significant differences in the sizes of anthropogenic particles within mussels compared to those in the overlying seawater at our study sites, with the average length of fibres in mussels significantly shorter than those in the seawater (One-way ANOVA; $F_{2, 745} = 10.270$, p -value < 0.001, Fig. 4a). A few longer fibres were found within a number of the mussels, with the longest fibre recorded being 8.7 mm in length, suggesting occasionally the longer fibres are ingested but this does not correlate to the proportions of longer fibres present in the overlying seawater. The average size of anthropogenic fragments ingested by mussels and found in the overlying seawater samples were also significantly smaller than the particles found within the surface sediment (One-way ANOVA; $F_{2, 54} = 47.710$, p -value < 0.001, Fig. 4b). Fibres made up 67.6% of the particles within mussel samples compared to 23.4% of those present in the water samples (One-way ANOVA; $F_{2, 140} = 11.795$, p -value < 0.001, Fig. 1). We found both high density and low density plastic polymers within the mussels, but the relative abundance of polymer types present differed from those found in the overlying seawater (Fig. 2).

We used a 50 µm mesh for our analysis across seawater, sediment and mussels here to enable a direct comparison of particles characteristics across these environmental compartments, and as such are likely missing particles present below this limit of detection. Whilst plastics are now being found at the nano-scale in the marine environment (Ter Halle et al., 2017) the sampling of open seawater with a limit of detection below 50 µm remains relatively rare and is an increasingly recognised gap in global marine plastics data. There is a trade-off when sampling surface waters of the volume of seawater that can be sampled versus limit of detection. Plankton nets of smaller mesh size clog rapidly with plankton and organic matter, and ‘whole water’ sampling methods tend to limit the volume of water that can be sampled to much smaller volumes. Sampling of sediments for the smaller microplastic fraction is even more challenging, particularly on beaches with fine sediment grain sizes. Hence the lower threshold of 50 µm used here was the lowest size threshold we could analyse quantitatively for reasonable sample volumes across all environmental compartments. The relationship between microplastic particles found within mussels and those in their surroundings may be quite different for particles smaller than 50 µm, however data on this size range of microplastic particles for coastal and open seawater is currently limited.

We found no significant correlation between number of particles in the overlying seawater samples and those found within the mussels at our given sites within our limit of detection, but did observe a significant positive correlation between number of particles in mussels and particles in their surrounding surface sediment (p -value = 0.031) (Fig. 5a & b). We found the proportion and the size range of fibres and the composition of the polymer types of these particles found within mussels to more closely reflect those found in the intertidal surface sediment compared to those found in the surface seawater (Figs. 1, 2 and 3). Small microplastic particles have been reported to have a lower rise velocity than large particles, resulting in greater susceptibility to

vertical transportation (Reisser et al., 2015). This may result in smaller particles remaining suspended within benthic water for a relatively longer period of time, increasing likelihood of encounter and uptake. Particle shape has also been shown to impact vertical transport and longevity of submersion of particles (Ballent et al., 2013), with films and “filaments” particularly susceptible to submersion by surface turbulence. Our findings contrast slightly with those reported by Qu et al. (2018), who found significant correlations between the abundance of surface seawater microplastics with mussel microplastic loads at sampling sites on the coast of China (coefficient of determination R^2 values between 0.44 and 0.75 were presented to support this relationship) and similar compositions of polymer types in the mussels and the overlying seawater. Sediments and beach debris were not sampled in the Qu et al. (2018) study. This disparity may be due to differences in the particle characteristics of the sea surface microplastics between the two studies sites. We observed a much higher proportion of microplastic fragments in our seawater surface tows (47% of sampled particles) compared to the Qu et al. study where fibres made up 90% of the microplastics in their seawater samples. Fibres dominate in the mussels in both studies, however, suggesting they are potentially more bioavailable to these benthic filter feeders. Since microfibrils are mostly modified cellulose (Rayon) this likely drives the similarity in polymer types between seawater and mussel microplastics in the Qu et al. (2018) study and explains the different relationship that we observe here when other polymer types are present in the overlying seawater. Differences in the habitat structure and/or coastal hydrodynamics of the regions sampled, as well as the abundances of plastics present and the distance to point sources of microplastics may also play a role in between site differences in this relationship. Additionally, the differing results may be the result of alternate methods of surface water sampling, Qu et al. (2018) used 5 L grab samples whereas we used plankton nets. Both methods have benefits and limitations (Barrows et al., 2017). Grab samples can capture the full range of particle sizes, but the small volume of water sampled may result in high variability between replicate samples. Plankton nets allow far greater volumes of water to be sampled efficiently, however are limited to a minimum particle size and will not capture all particles.

The differences in both the size range and the polymer composition of the plastics found within the mussels compared to their overlying seawater and surrounding beach sediments, suggest that uptake of microplastics into mussels may not always directly proportional to what is in their surrounding environment. It is likely that both environmental and biological partitioning of microplastic particles and the selective feeding ecology of this species is responsible for the under-representation of certain polymer types and particle sizes within the mussels. Bivalves have feeding mechanisms which enable them to discard larger particles as pseudofaeces prior to ingestion (Defosse and Hawkins, 1997). Indeed Kolandhasamy et al. (2018) found that the largest microplastics in their study were adhered to the foot and mantle rather than ingested and so the capture of particles by feeding structures in suspension feeders such as mussels is the product of particle encounter rates and retention (Shimeta and Jumars, 1991). It is likely that a range of factors influence mussel encounter rates with particles within their immediate environment, including particle behaviour in the water column and small scale hydrodynamics. Fibrous particles may have a greater tendency for entanglement within complex feeding structures and potentially even be retained for longer periods within the gut once ingested (Kolandhasamy et al., 2018; Murray and Cowie, 2011). Preferential retention of certain shapes of particles may then indirectly influence the types of polymers found within *M. edulis*, since the majority of fibres in our samples were cellulose. Some polymer types were under-represented or totally absent in the mussels compared to overlying seawater or surrounding sediment. This should be taken into consideration when using mussels as bioindicators of plastic pollution, since microplastic particles and polymer types that may pose a risk to other biota with differing feeding modes might be missed if this were

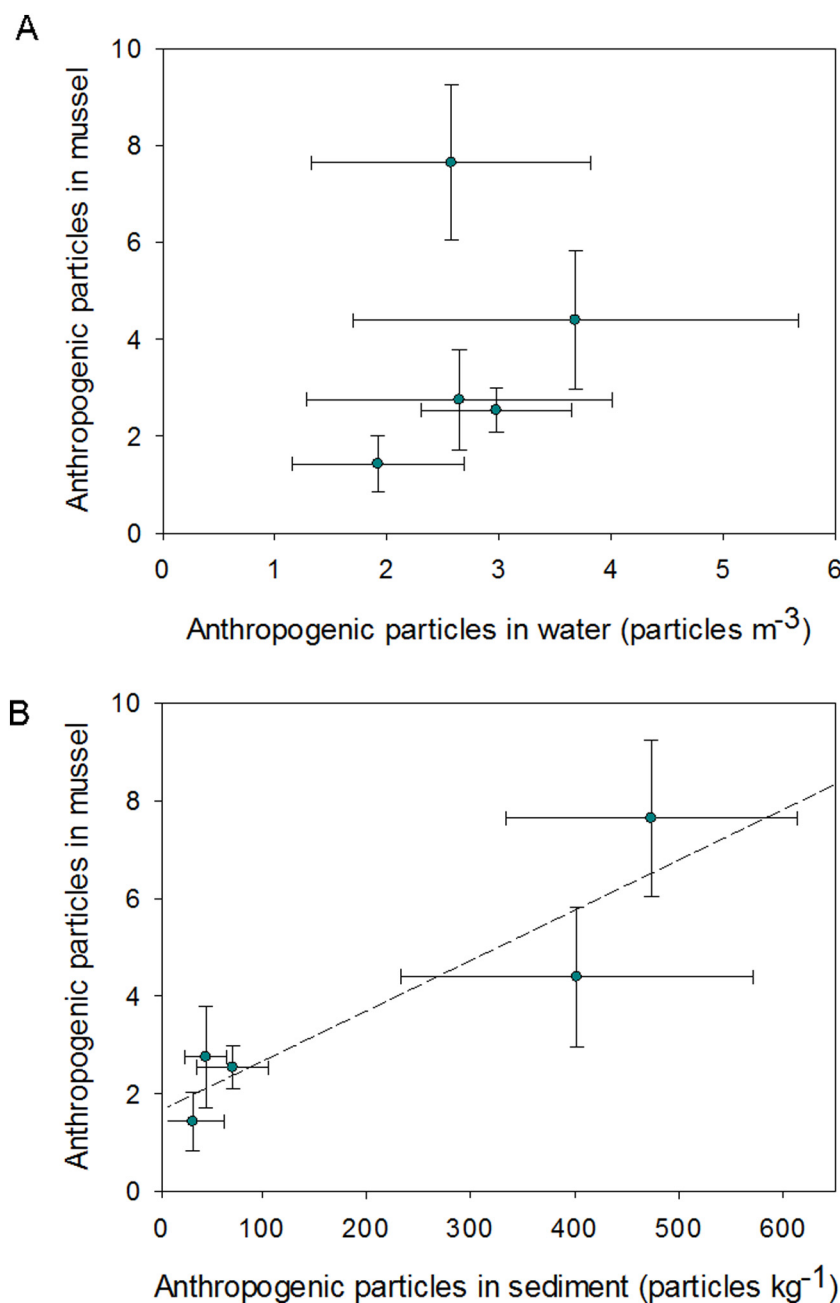


Fig. 5. The number of anthropogenic particles in *Mytilus edulis* compared to those in (A) the overlying seawater and (B) the surface 1 cm of sediment at coastal sites across Devon and Cornwall, SW England. Regression line shown as dashed lines (Linear regression, $R^2 = 0.832$, $F(1, 3) = 14.870$, p -value = 0.031).

the only monitoring tool used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2019.05.041>.

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